Epstein-Barr Virus-associated Smooth Muscle Tumors in AIDS Patients: A Largest Case (Series)

Ratima Issarachaikul\textsuperscript{1}, Shanop Shuangshoti\textsuperscript{2} and Chusana Suankratay\textsuperscript{1}

Abstract

This study aimed to determine the outcomes of Epstein-Barr virus (EBV)-associated smooth muscle tumors (SMTs) in AIDS patients at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, treated from 2001-2011. Of the 17 patients, there were five men with a median CD4 count of 26 cells/μL. Eight and nine patients had single and multiple sites, respectively. The most common site was the cranial epidura (58.8%). All patients had EBV within the tumor. Seven patients underwent surgery. The median follow-up was one year. The mortality rate was 41.2%. All patients with undetectable HIV viremia survived. This is the largest case series regarding EBV-associated SMTs in AIDS patients with a long follow-up period.

Key words: Epstein-Barr virus, smooth muscle tumors, AIDS, HIV

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Introduction

Epstein-Barr virus (EBV), a member of the herpesvirus family, has been documented to be associated with several kinds of tumor, including Hodgkin’s lymphoma, non-Hodgkin’s lymphoma and nasopharyngeal carcinoma, in both immunocompetent and immunocompromised individuals (1-3). Several reports have described the association between EBV and the occurrence of smooth muscle tumors (SMTs), especially in immunocompromised patients, including transplant recipients and AIDS patients (2, 4-6). It is likely that immunosuppression can predispose patients to the development of SMTs (7). The association became more evident as the number of AIDS patients and organ transplant recipients has significantly increased in the past few decades (4, 5, 8-10).

Our previous study also showed evidence of an association between EBV infection and SMTs in all nine AIDS patients (11). Most patients had fatal outcomes despite complete tumor removal. However, there were only a few patients receiving combination antiretroviral therapy (cART) at that time. The present study thus aimed to confirm the association of EBV infection with SMTs, as well as to determine the outcome in a larger number of AIDS patients treated during the cART era and with a longer follow-up period.

Case Report

Study design

A retrospective study was carried out in all 17 HIV-infected patients with SMTs treated at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from January 1, 2001 to December 31, 2011. Patients A total of 17 AIDS patients (16 adults and one child) with SMTs were included in the present study (Table). All data, including data related to the epidemiology, clinical manifestations, treatment, and outcome were evaluated. Blood samples were assayed for the EBV and HIV viral load by quantitative polymerase chain reaction (PCR), and serum samples were subjected to an EBV serological analysis at the time of diagnosis. Tumor tissues were tested for SMTs and EBV by standard immunohistochemistry and in situ hybridization.

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<th>EBER-1</th>
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Plasma and tissue studies

The plasma HIV viral load was determined using the HIV-1 RNA Monitor Assay, version 2 (Roche Molecular Systems, Inc., Plesanton, USA), which had a lower limit of detection of 40 copies/mL.

The plasma EBV viral load was determined by real-time quantitative PCR, which was carried out according to the previously reported method (12). In brief, 400 hundred μL of plasma samples were extracted with a QIAamp Blood kit (Qiagen, Valencia, U.S.A.), with a final elution of 50 μL, and then the EBV DNA was detected by real-time quantitative PCR targeting the BamHI-W fragment region of the EBV genome.

For the EBV serologic testing, the serum anti-EBV viral capsid antigen (VCA) IgG and IgM levels, as well as the anti-EBV nuclear antigen (EBNA) IgG levels, were measured using standard enzyme-linked immunosorbent assay (ELISA)s (Dia Pro Diagnostic Bioprobes, Milan, Italy).

For the light microscopy studies, formalin-fixed, paraffin-embedded sections of the tumor were stained with hematoxylin and eosin, and were analyzed for the cellular morphology and mitotic activity. The number of mitoses was derived from a count of dividing cells in 10 high-power fields (HPFs) in the most mitotically active area.

The immunohistochemical studies used formalin-fixed, paraffin-embedded sections of the tumor that were deparaffinized, rehydrated and immunostained using the standard streptavidin-biotin-peroxidase method, with 3,3'- diaminobenzidine used as the chromogen. Stained slides were counterstained with hematoxylin. Smooth muscle actin (Bio Genex, dilution 1: 200) and desmin (Dako, dilution 1: 200) immunostaining were carried out for all samples. Staining for the S-100 protein (Dako, dilution 1: 200), HMB-45 (Dako, dilution 1: 50), epithelial membrane antigen (Dako, dilution 1: 50), CD34 (Bio Genex, dilution 1: 50), and CD 99 (Dako, dilution 1: 10) was carried out when there was a need to exclude the histologic mimics of SMTs.

For in situ hybridization, formalin-fixed, paraffin-embedded sections of the tumor were dewaxed, rehydrated and pretreated with proteinase K. Hybridization with EBV-encoded small RNA-1 (EBER-1) was carried out using a fluorescein-conjugated EBV oligonucleotide probe (Novocastra EBV Polyprobe kit) at 37°C for two hours. The detection of the signal was performed by placing slides in a solution containing the rabbit F(ab')2 alkaline phosphatase-conjugated antibody to fluorescein isothiocyanate, and then in alkaline phosphatase substrate buffer (pH 9.0). The alkaline phosphatase activity was assayed using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. Stained slides were counterstained with hematoxylin.

Results

Patient demographics

Seventeen AIDS patients with SMTs were identified during the study period (Table). Patients 1-8 and 11 have been described elsewhere (11). The study patients included 16 adults and 1 child (5 men and 12 women). The mean age was 34+9.2 years. All adult patients had heterosexually acquired HIV infection. Apart from two patients with SMTs as the first presentation, the median duration of diagnosis of HIV infection before the diagnosis of the SMTs was four years, with an interquartile range (IQR) of 2-6.5 years. At the time of the diagnosis of the SMTs, the median CD4 count was 26 cells/μL, with an IQR of 9.75-154.5 cells/μL, and four patients had an undetectable of HIV viral load. Eight (47.1%) patients had received cART. By the end of the study, seven patients had died and 10 patients had survived.

Clinical characteristics

There were eight and nine patients with single and multiple tumor sites, respectively. The most common site was the cranial epidura (10 patients, 58.8%) (Fig. 1), followed by the spinal epidura (seven, 41.2%), lung, liver, vocal cord, abdominal wall and adrenal gland (two patients each), pleura, kidney, thigh, orbit and iris (one patient each). Of the nine patients with multicentric SMTs, there were three (33.3%), four (44.5%), and two (22.2%) patients with central nervous system (CNS) only, CNS and extra-CNS and extra-CNS only tumors, respectively. All patients had evidence of EBV infection in the tumor, as demonstrated by positive EBER-1 in situ hybridization, and two patients had detectable blood EBV viral load.

Histopathology

On hematoxylin and eosin staining, all SMTs showed interlacing fascicles of spindle-shaped smooth muscle cells (Fig. 2). The tumor nuclei showed mild-to-moderate pleomorphism. The maximum number of mitoses per 10 HPFs varied significantly, being >10 in three patients; one to three in nine patients; zero in one patient, and no data were available in four patients. All SMT specimens showed strong nu-
clear staining for EBER-1 in situ hybridization.

Virology

Eight plasma samples were evaluated to determine the EBV viral load using real-time quantitative PCR, and two samples were positive, with plasma EBV DNA levels of 22.5 and 45,108 copies/mL (Table).

EBV serology

Serum samples were assayed from 15 patients. All showed evidence of past EBV infection, as demonstrated by the positive EBV VCA IgG and EBNA IgG in association with negative EBV VCA IgM (Table).

Treatment

Seven (41.2%), seven (41.2%), and three (17.6%) patients received surgery, both surgery and radiotherapy and neither surgery nor radiotherapy, respectively. At the time of the diagnosis of SMTs, five patients who were naïve to antiretroviral therapy began to receive cART, two patients had changed cART regimen due to virological failure and eight patients had continued to receive cART (no data available for two patients).

Outcomes

The median follow-up duration was one year, with an IQR of 0.5-5.5 years. The overall mortality rate was 41.2%; all seven patients with a detectable HIV viral load died. In contrast, all 10 patients with an undetectable HIV viral load and immunological improvement survived, despite incomplete tumor removal in some patients. In the seven patients who died, the median CD4 cell count was 15 cells/μL, with an IQR of 7-367 cells/μL. In the 10 surviving patients, the median CD4 cell count was 421 cells/μL, with an IQR of 319-521 cells/μL.

Discussion

Several previous studies have reported an increased frequency of certain tumors, including non-Hodgkin’s lymphoma, cervical carcinoma, and Kaposi sarcoma, in AIDS patients (13-15). However, the increased incidence of EBV-associated SMTs in AIDS patients has been documented more recently (4, 5, 8-10). The present study provides additional confirmation of the association between EBV infection and SMTs in AIDS patients. The majority of our patients were in the advanced stage of HIV infection, with a median CD4 count of 26 cells/μL. This observation is consistent with recent reviews (6, 9, 10). In addition, our patients were relatively young, with a mean age of 34±9.2 years, also consistent with the previous reviews (6, 9, 10).

In the present study, EBV-associated SMTs had unique clinical features including multicentric involvement either concurrently or sequentially, a predilection for the cranial and spinal epidural and unusual extra-CNS locations (lung, liver, vocal cord, abdominal wall and adrenal gland), which were consistent with our previous study (11). A recent review by Purgina and colleagues also showed that multifocal tumors and CNS involvement were very common in EBV-associated SMTs in AIDS patients (9). In contrast, SMTs are generally unifocal in immunocompetent hosts. The CNS, including both the cranial and spinal epidural, is the most common site of involvement of SMTs in AIDS patients (9-11, 16, 17). Primary CNS SMTs are unusual. Only five and three patients with cranial and spinal epidural have been reported in the literature, and five of these patients were infected with HIV (11, 18). In the present study, all SMTs in the brain were enhancing epidural masses located in the sellar and/or parasellar areas, consistent with other reports (6, 9-11, 18). In addition, the majority of our patients with spinal SMTs presented with epidural masses in the neural foramina, mimicking nerve-sheath tumors. SMTs should therefore be included in the differential diagnosis of cranial or spinal epidural masses, especially in AIDS patients with multiple sites of involvement.

In the present study, the second most common site of EBV-associated SMTs in AIDS patients were the lung, liver, vocal cord, abdominal wall and adrenal gland, which are rarely observed in non-HIV-infected patients (19, 20). These observations are consistent with those of three recent reviews (6, 9, 10).

In the present study, most EBV-associated SMTs were histologically well differentiated tumors with a mild degree of polymorphism of the nuclei and a low level of mitotic activity, which is consistent with previous reviews (6, 9, 11). However, it is very difficult to determine whether these tumors are benign or malignant. Definite criteria for distinguishing between benign and malignant neoplasm outside the genitourinary and gastrointestinal tracts have not been established (21). This problem is further complicated by the immunocompromised status of the all AIDS patients with
very low CD4 cell counts. In addition, it has been postulated that the aggressiveness of SMTs in immunocompromised individuals is determined by the immune condition, rather than by histological features (22). Moreover, a recent molecular study by Deyrup and colleagues has documented that the tumors located at each site were derived from a different clone of EBV, compatible with multiple independent primary tumors, rather than metastasis (6).

The present study confirmed the presence of EBV in all patients with SMTs. However, only two of the eight plasma samples evaluated were positive for EBV by real-time quantitative PCR, thus suggesting that this test has the low sensitivity for diagnosing and assessing the outcomes of treatment of EBV-associated SMTs in AIDS patients. This is in contrast with the very high sensitivity of the test for the diagnosis of nasopharyngeal carcinoma (23).

In the present study, all seven patients with virological failure on cART died. In contrast, all 10 patients with both virological and immunological responses to cART survived despite incomplete tumor removal in some patients. At present, there is no standard treatment for EBV-associated SMTs in AIDS patients. A recent review by Yin and colleagues described that there have been no randomized controlled studies for treating EBV-associated SMTs in pediatric AIDS patients, and hence, they recommend a case-by-case assessment of the immune status by cART, rather than by histological features (22). Moreover, a recent molecular study by Deyrup and colleagues has documented that improvement of the immune status by cART, analogous to the treatment of EBV-associated PTLDs (26-28). This appears to be consistent with the present study showing improved survival in all of the AIDS patients with virological and immunological responses to cART, despite incomplete tumor removal in some patients.

In conclusion, to the best of our knowledge, this is the largest case series which has analyzed EBV-associated SMTs in AIDS patients both in the pre-cART and cART era, with a long follow-up period. The present study suggests that improvement of the immune status by cART, analogous to the treatment of EBV-associated PTLDs, will result in better outcome for EBV-associated SMTs in AIDS patients.

The authors state that they have no Conflict of Interest (COI).

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